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THE FORMATION OF CHLAMYDOSPORES IN SPOROTHRIX SCHENCKII *

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The statement has been made repeatedly by French workers, especially by Beurmann and Gougerot, that *Sporothrix schenckii* does not produce chlamydospores, whereas *Sporothrix beurmanni* does. This has been indeed one of their most important points of differentiation in their attempt to establish the existence of two distinct pathogenic sporothrices.

In his article on "Sporotrichosis" Gougerot,[1] referring to the sporothrix beurmanni, writes:

"Chlamydosporen von verschiedener Grösse und Gestalt. Sie konnen 4- 16- 18 μ lang werden, sind rundlich oder gestreckt, in der Länge des Fadens verteilt oder an dessen Ende, isoliert oder in Gruppen odor in Ketten von 2, 3 oder mehr Gliedern. Sie enstehen auf dem Fadern selber oder auf einem kurzen besonderen Ast." Referring to the sporothrix schenckii, he writes: "Keine Chlamydosporen." Gougerot, in his paper on "North American Sporotrichosis,"[2] quotes Matruchot, the eminent French botanist, as follows: "Le Sporotrichium Beurmanni peut donner naissance a des chlamydospores, c'est-a-dire à des spores enkystées, munies d'une membrane èpaisse. De Beurmann et Gougerot en avaiant deja signalé d'intercalaires sur le trajet des filaments mycelium et de terminales a l'extrémité de rameaux lateraux renflés; nous avons pu, nous aussi, en observer de laterales et de terminales, peut être plus caractéristiques que les intercalaires par leur forme subsphérique et par les gouttelettes huileuses qu'elles renferment. Elles apparaissent dans les vielles cultures, dans les points où se produisent de petites touffes floconneuses de mycélium blanc; elles sont abondantes, surtout sur les portions agregées du mycélium; ce sont evidemment des organes de conservation." Referring to the sporothrix schenckii, he writes: "Pas des chlamydospores connues."

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* Received for publication August 15, 1914.

[1] Kolle and Wassermann Handbuch der Pathogenen Micro-organism, 2d edition, 1913, 5, p. 232.

[2] Bull. et mém. Soc. méd. d. hôp. de Paris, 1910.

The strain of *Sporothrix schenckii,* referred to in these references, was sent by Hektoen to Beurmann in 1906. The observations which I wish to report in this paper were made on the same organism, a culture of which I received from Hektoen in 1910.

In the original articles by Schenck[3] and by Hektoen[4] no mention is made concerning the existence or non-existence of chlamydospores. We may safely assume that they were not observed, since later studies have shown that in ordinary nutrient media they are not found in cultures of the original Hektoen strain. I wish to point out here that this strain is not unique in this respect, since, as I will show later, other strains exist which likewise do not produce chlamydospores on ordi-

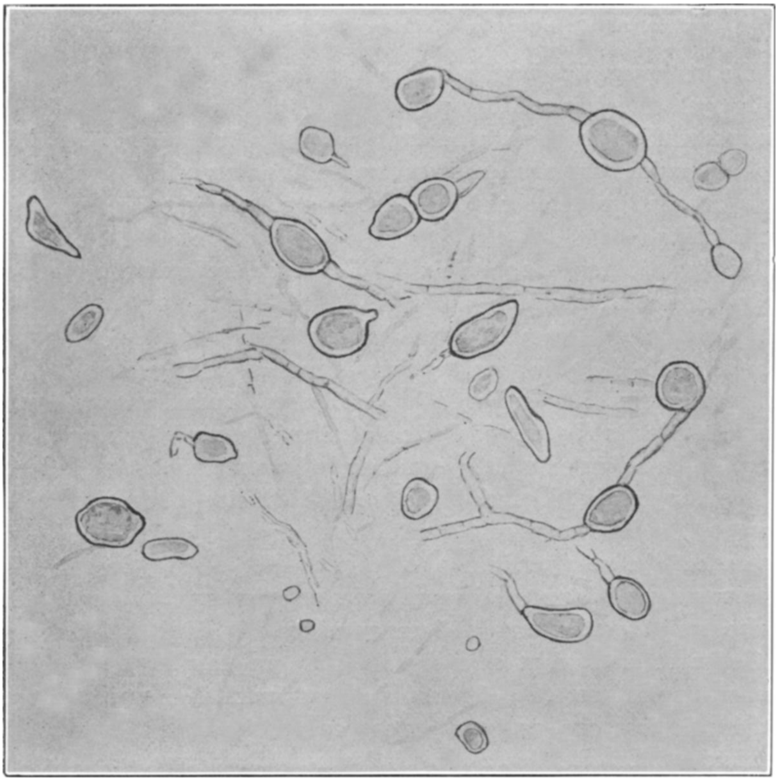

Fig. 1.—Chlamydospores of Sporothrix schenckii grown on agar 1.5 percent, NaCl 0.5 percent and water for eight weeks.

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[3] Bull. Johns Hopkins Hospital, 1898, 9, p. 286.
[4] Jour. of Exp. Med., 1900, 5, p. 77.

nary nutrient media. Certain strains, however, produce them in moderate numbers and it is this variation in strains on ordinary media that has evidently given rise to such statements as those quoted.

While observing the growth on different media of sporothrix, including, among others. strains obtained from Gougerot and Sabaraud as typical *Sporothrix beurmanni* and also the original Hektoen strain, I noted that on media poor in nutrients such, for example, as agar 1.5 percent, NaCl 0.5 percent, with or without sugar, the formation of chlamydospores was favored very decidedly. I was led, therefore, to look into this matter rather carefully, and with this point in mind a number of strains, grown on various media both rich and poor in nutrients, were examined. The strains vary considerably. Some strains, for example, a sporothrix obtained from Gougerot, one from Kren of Vienna, and one from K. F. Meyer isolated from a horse in Pennsylvania, show, on ordinary nutrient glucose agar, the presence of an occasional chlamydospore. Others, like the original Hektoen strain and a sporothrix obtained from Sabaraud in Paris, apparently do not form them in nutrient media or at least only very rarely. However, in media poor in nutrients the number of chlamydospores is very much increased. They have been observed to be numerous in the following media: Agar 1.5 + Na Cl .5; agar 1.5 + asparagin 2 + $MgSO_4$ 1 + K_2HPO_4 1; either of the above with the addition of sugars, pure or impure. I have made careful notes on the eight strains which I have in my possession, and in all the formation of chlamydospores was abundant when placed on these media. There were some variations in the number of spores in the various tubes between the strains, but these were not constant; nor did they indicate any characteristic differences between the strains which could be interpreted as meaning a variety difference. I wish to suggest here that those who may have occasion to study the properties of sporothrix strains should observe whether chlamydospore formation occurs on the synthetic media mentioned.

The chlamydospores vary considerably in size, ranging from 5 microns in diameter to many times this dimension. Some appear spherical. others oblong; some are irregular and appear as tho plasmolyzed and shrunken. They are usually colorless, or only very faintly yellow. I have not seen definitely pigmented ones as the ordinary spores often appear. They occur at times in the course of a filament; again at its terminus. They are also occasionally seen attached to the side of a mycelial thread by means of a fine stalk much as are the ordinary spores. The walls are usually thick and often irregular. They stain deeply with methylene-blue and are strongly gram-positive. Their shape is depicted in Figure 1, which is a drawing from an unstained preparation made from an eight-weeks old culture of the sporothrix schenckii. This is the strain that was obtained from Hektoen and which Gougerot has declared does not form chlamydospores.

The chlamydospores form apparently rather deep in the media where there are few or no ordinary spores, but where the mycelium is abundant. It does not appear that the oxygen supply has any material influence on their formation, for organisms grown in a limited supply of oxygen do not seem to form them more readily than when air is freely admitted. Furthermore, organisms grown on nutrient media which has been permitted to dry does not, as one might suppose, favor their formation.

It is a matter of interest that when sporothrix is planted on a medium of low nutrient value, the growth, instead of remaining largely at the surface, will penetrate deep into the culture, in time often growing well to the bottom of the tube and throughout the entire medium. It is in this mass, which can be readily seen in the media as a diffuse growth, that chlamydospore formation goes on apparently in greatest abundance. The spores may be very numerous here, at times a dozen or more being seen in a single field of the microscope.

The chlamydospores do not form in the media for some little time after distinct growth is visible. A series of tubes of the various synthetic media referred to above were inoculated and examined from time to time. After ten days a few were noted in an occasional tube; in some tubes there were none. From this time on, they gradually appeared in increasing numbers, and at the end of one month every tube contained them, some in very large numbers.

SUMMARY

The sporothrix schenckii in certain synthetic media readily forms typical chlamydospores indistinguishable from those seen in cultures of the sporothrix beurmanni.

Considerable variation in respect to chlamydospore formation exists between the various strains of sporothrices when grown on ordinary nutrient media. They all readily form them, however, when grown on synthetic media poor in nutrients.

Chlamydospore formation cannot, therefore, serve as a differentiating point between *Sporothrix schenckii* and *Sporothrix beurmanni.*